EPA Reviewer: Lisa Austin, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/30/09
Template version 02/06

TXR#: 0055057

HED Executive Summary Cover for the attached OECD Formatted DATA EVALUATION RECORD

STUDY TYPE: Subchronic (90-Day Oral) Toxicity [feeding, capsule]-[dog]; OPPTS 870.3150 [§82-1b] (non-rodent); OECD 409.

PC CODE: 118203 **DP BARCODE:** D349929

TEST MATERIAL (PURITY): BAS 800 H (94.2%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449, saflufenacil

CITATION: Kaspers, U., Deckardt, K., Burkhardt, S. et al. (2006) BAS 800 H – Repeated dose 90-day oral toxicity study in Beagle dogs administration via gelatin capsules. Experimental Toxicology and Ecology, BASF AG, Ludwigshafen, FGR. Report Number(s) 41D0414/01182. April 5, 2006. MRID 47128113. Unpublished.

SPONSOR: BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, FRG.

EXECUTIVE SUMMARY:

In a 90-day toxicity study (MRID 47128113), BAS 800 H (94.2%, Lot#, COD - 000606) was administered daily via gelatin capsules to purebred Beagle dogs, 5/sex/group, at nominal doses of 0, 10, 50, or 150 mg/kg bw/d.

There were no treatment-related effects on mortality, ophthalmoscopy, urinalysis, or gross pathology. Signs of systemic toxicity were evident at 50 and 150 mg/kg bw/d.

At 50 mg/kg bw/d, BAS 800 H resulted in decreased mean corpuscular volume (MCV, 4-7%), mean corpuscular hemoglobin (MCH, 6-8%) and histopathological findings in the liver (iron storage, 3/5 vs 0/5 controls).

At 150 mg/kg bw/d, BAS 800 H lowered body weight (6-7%) and body weight gains (73-110%), slightly decreased food consumption and food efficiency, increased the incidence of dark brown/dark red brown feces (5/5 vs 0/5 controls), changes in hematological parameters associated with moderate-to-severe anemia (decreased values in hemoglobin (14-19%) levels, Hct (10-15%), MCV (9-24%), MCH (13-27%), and mean corpuscular hemoglobin concentration (MCHC, 2-5%) as well as histopathological findings in the liver (iron storage, 4-5/5 vs 0/5 controls), spleen (extramedullary hematopoiesis, 2/5 females vs 0/5 controls) and sternum hyperplasia (2/5 both sexes vs 0/5 controls) and bone marrow (hyperplasia, 2/5 females vs 0/5 controls).

The LOAEL was 50 mg/kg bw/d based on lower MCV and MCH values and increased iron storage in the liver in both sexes and the NOAEL was 10 mg/kg bw/d.

This 90-day oral toxicity study in the dog is acceptable guideline and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in dogs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and Data Confidentiality statements were provided.

This Executive Summary was prepared for the United States Environmental Protection Agency, Office of Pesticide Program, Health Effects Division Use.

Much of the text was generated by the submitter(s) in OECD format. However, this document has undergone critical scientific analysis in comparison to the study report and modified as needed.

Reviewer #: Steve Wong, Ph.D. , Date April 30, 2008

APPLICANT: BASF Corporation

STUDY TYPE: 90-Day oral toxicity in dog; administration via capsule; OPPTS 870.3150 (non-rodent); OECD 409.

TEST MATERIAL (PURITY): BAS 800 H (93.8%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449

CITATION: Kaspers, U., Deckardt, K., Burkhardt, S. et al. (2006) BAS 800 H – Repeated dose 90-day oral toxicity study in Beagle dogs administration via gelatin capsules. Experimental Toxicology and Ecology, BASF AG, Ludwigshafen, FGR. Report Number(s) 41D0414/01182. BASF Doc ID 2006/1007441. April 5, 2006. Unpublished. [PMRA # 1547023]

SPONSOR: BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, FRG

EXECUTIVE SUMMARY:

In a 90-day toxicity study, BAS 800 H (93.8%) was administered daily via gelatine capsules to purebred Beagle dogs, 5/sex/group, at 0, 10, 50, or 150 mg/kg bw/d. There were no treatment-related effects on mortality, ophthalmoscopy, urinalysis, or gross pathology. Signs of systemic toxicity were evident at 50 and 150 mg/kg bw/d. At 150 mg/kg bw/d, BAS 800 H induced lower body weight and body weight gains, decreased food consumption and food efficiency, dark brown/dark red brown feces, changes in hematological parameters associated with moderate-to-severe anemia (decreased values in haemoglobin levels, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) as well as histopathological findings in the liver (iron storage), spleen (extramedullary hematopoiesis) and bone marrow (hypertrophy). At 50 mg/kg bw/d, the treatment-related findings were lower MCV and MCH values in both sexes. The LOAEL was 50 mg/kg bw/d and the NOAEL was 10 mg/kg bw/d.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

65

I. MATERIALS AND METHODS

A. MATERIALS:

1.

Test material:	BAS 800 H
Description:	Solid / bright-beige; stored at room temperature
Lot/Batch #:	COD - 000606
Purity:	93.8% a.i.
Compound stability:	The stability under the storage conditions present in this study was guaranteed by the Certificate of Analysis. The homogeneity of the test material was confirmed by analysis.
CAS #:	372137-35-4

2. **Vehicle and/or positive control:** BAS 800 H was administered via gelatine capsules.

3. **Test animals:**

Species:	Dog	
Strain:	Purebred Beagle	
Age/weight at study initiation:	Age: 7 to 8 months Body weight: ♂ = 14.6 (12.4– 6.7); ♀ = 12.7 (10.7–15.3) kg	
Source:	BASF Beagle Colony	
Housing:	1. Up to study day -1: Building Z455; floor area ~5.4 m^2 (inner kennel ~2.7 m^2; outer kennel ~2.7 m^2) 2. Post study day -1: Building Z457; floor area about 6 m^2 (inner kennel ~1.5 m^2; outer kennel ~4.5 m^2) There was one dog per kennel. The dogs had day-and-night access to the outer kennel.	
Diet:	Dog maintenance KLIBA laboratory diet (pellets); Switzerland; About 400g/day for a period of 2 hours. Any left over food was weighed and subtracted from the amount of food offered.	
Water:	Demineralized water, adjusted with drinking water to about 2° hardness; *ad libitum*	
Vaccination	Distemper, hepatitis, leptospirosis, parvovirus, rabies and deworming at regular intervals	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	Heating of the air supply was provided in the winter Ambient humidity Ventilation by forced ventilation system Natural day/night cycle with artificial light as required during working hours
Acclimation period:	At least seven days prior to application	

B. STUDY DESIGN:

1. **In life dates:** Start: July 5, 2005 End: October 14, 2005

2. **Animal assignment:** Animals were assigned to test groups via a randomization protocol provided by a computer. The test groups are noted in Table 1.

Table 1: Study design

	♂				♀			
mg/kg bw/d	0	10	50	150	0	10	50	150
N	5	5	5	5	5	5	5	5

3. Dose preparation and analysis:

The appropriate amounts of BAS 800 H, adjusted on the basis of individual animal's weekly body weight, was weighed and placed in gelatine capsules (stomach-soluble hard gelatine capsules). The prepared capsules were stored at room temperature.

4. Statistics:

Parameter	Statistical test*	Reference
Food consumption, body weight, body weight change	A comparison of each group with the control group using the Dunnett-test (2-sided) for the hypothesis of equal means	Winer, B.J. (1971): Statistical principles in experimental design. McGraw-Hill New York, 2nd edition. Dunnett, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 - 1121 Dunnett, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 - 491
Clinical pathology parameters, except reticulocytes and differential blood count	Non-parametric one-way analysis using Kruskal-Wallis test (2-sided).If p≤0.05, a pair-wise comparison of each dose group with the control group was performed using Wilcoxon-test (2-sided) for the equal medians	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York
Urinalysis, except volume, color, turbidity and specific gravity	Pair-wise comparison of each dose group with the control group using Fisher's exact test for the hypothesis of equal proportions	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York
* Significantly different (p <0.05) from the control; ** Significantly different (p <0.01) from the control		

C. METHODS

1. Observations:

The dogs were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Detailed clinical observations were conducted for all animals prior to the administration period and thereafter at weekly intervals. Parameters examined were as follows:

activity / arousal level	skin	tremors	lacrimation	fur	mucosal membranes
abnormal behaviour during handling	feces (appearance /consistency)	abnormal movements	impairment of gait	pupil size	visible swellings / masses
posture	salivation	convulsions	respiration	urine	

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter.

3. Food consumption:

Food intake was determined each working day, starting on day -7 (beginning of the adaptation period) and calculated as mean food consumption in grams per dog per day. The dogs were offered food before the

administration of the gelatin capsules for a period of up to two hours. Any food left over was weighed thereafter and subtracted from the amount of food offered. Food efficiency was calculated for each animal at weekly intervals on the basis of body weight changes and the total amount of food consumed during this period, using the formula below:

$$\frac{BW_x - BW_{x-7}}{FC} \times 100$$

BW_x = Body weight on day x (in g)
BW_{x-7} = Body weight on day x - 7 (in g)
FC = Total daily food consumption (in g) from day x-7 to day x-1

4. Ophthalmoscopic examination:

All dogs were examined with an ophthalmoscope prior to and at the end of the administration period.

5. Hematology & clinical chemistry:

Blood was removed from non-anesthetised, fasted animals from the vena cephalica antebrachii. The blood was withdrawn at three separate time points in the study: prior to the beginning of the experiment (D14 to D13); at the middle of the experiment (D41 to D43); and at the end of the study (D93 to D94). The checked (x) parameters were examined.

a. Hematology:

x	hematocrit (Hct)*	x	leukocyte differential count*	x	reticulocyte count
x	hemoglobin (Hb)*	x	mean corpuscular Hb (MCH)	x	platelet count
x	leukocyte count (WBC)*	x	mean corpuscular Hb concentration(MCHC)		
x	erythrocyte count (RBC)*	x	mean corpuscular volume (MCV)		
x	blood clotting measurements*, prothrombin time (thromboblastin time; clotting time)				
* Recommended by OECD 407 and US EPA Guideline 870.1350					

b. Clinical chemistry:

Electrolytes				Others			
x	calcium*	x	sodium*	x	total protein (TP)*	x	total cholesterol
x	chloride*	x	potassium*	x	albumin*		blood creatinine*
x	magnesium	x	phosphorus*	x	globulins	x	blood urea nitrogen*
Enzymes				x	glucose*	x	total bilirubin*
x	alkaline phosphatase (AP)			x	triglycerides		serum protein electrophores
x	serum alanine amino-transferase (ALT/SGPT)*					x	total porphyrins in plasma
x	serum aspartate amino-transferase (AST/ SGOT)*					x	total porphyrins in urine
	creatine phosphokinase					x	total porphyrins in feces
x	gamma glutamyl transferase (GGT)*						
	cholinesterase (ChE)						
	lactic acid dehydrogenase (LDH)						
	glutamate dehydrogenase			* Recommended by OECD 407 and US EPA Guideline 870.1350			
	ornithine decarboxylase*						

6. Urinalysis:

Urine was collected at days 11-12, 44-45, and 86-87 for urinalysis. For urine collection, individual animals were transferred to metabolism cages (food withdrawn, about 500 mL of water), and urine was collected overnight. The following parameters (x) were analyzed:

x	volume*	x	specific gravity*	x	glucose*	x	urobilinogen	x	ketones	x	sediment
x	pH*	x	color, turbidity	x	protein*	x	blood*	x	bilirubin	x	appearance
* Recommended for subchronic non-rodent studies based on Guideline 870.1350											

7. Sacrifice and pathology:

All dogs that died and those sacrificed on schedule (anesthetized and sacrificed by exsanguination from the cervical and brachial vessels) were subjected to gross pathological examination and the checked (x) tissues were collected for histological examination. The (xx) organs were weighed.

Digestive system				Cardiovacular/hematological				Neurological	
	tongue	x	cecum*	x	aorta*	x	bone marrow*	xx	brain*+
x	salivary glands*	x	colon*	xx	heart*+	x	lymph nodes*	xx	pituitary*
x	esophagus*	x	rectum*	xx	spleen*+	xx	thymus*+	x	sciatic nerve*
x	stomach*	xx	liver*+	Urogenital				x	spinal cord (3 levels)*
x	duodenum*	x	gallbladder*+	xx	kidneys*+			x	eyes (optic nerve)*
x	jejunum*	x	pancreas*	x	urinary bladder*			Glandular	
x	ileum*			xx	testes*+			xx	adrenal gland*+
Respiratory				xx	epididymides*+			x	mammary gland*
x	trachea*	x	nose*	xx	prostate*			x	parathyroids*+
x	lung	x	pharynx*	x	seminal vesicle			x	thyroids*+
x	nasal cavity	x	larynx*	xx	ovaries*+				lacrimal gland
				xx	uterus*+ and vagina				
Others									
x	bone	x	skin	x	gross lesions and masses*			x	target organs*
				x	skeletal muscle				
* Recommended by OECD 407 and US EPA Guideline 870.1350; + Organ weight required for non-rodent studies by OECD 407									

II. RESULTS

A. Observations:

1. Clinical signs of toxicity:

Dark brown/dark red brown discoloured feces were seen in all dogs at 150 mg/kg bw/d. This finding was likely caused by excretion of prophyrins via feces, due to the mode of action of BAS 800 H as a protoporphryinogen IX oxidase inhibitor. There were no other treatment-related findings.

2. Mortality: All dogs survived the study period.

B. Body weight and weight gain:

Although there was no statistically significant deviation of the body weight in any test group (males and females) in comparison to the control groups. Body weights of high-dose dogs were consistently lower than those of the control dogs; from day 35 to 91 in males and for the entire dosing period in females. The lower body weights of high-dose groups were considered treatment induced adverse effects. Body weights and body-weight gains of dogs at ≤50 mg/kg bw/d were not affected.

Table 2. Body weight and body-weight gain data, kg±SD

	♂ (N = 5/group)				♀ (N = 5/group)			
mg/kg bw/d	0	10	50	150	0	10	50	150
Day -1	14.5±1.2	14.8±1.3	14.4±1.4	14.5±1.4	12.5±0.8	12.8±1.4	13.0±1.5	12.5±1.3
Day 7	14.6±1.1	15.0±1.3	14.7±1.3	14.7±1.4	12.7±0.8	13.0±1.5	13.2±1.5	12.4±1.2
Day 28	14.8±0.9	15.2±1.3	15.1±1.1	14.8±1.3	12.8±0.8	13.1±1.4	13.3±1.5	12.5±0.9
Day 35	14.7±0.9	15.1±1.2	15.1±1.1	14.6±1.2	12.9±0.9	13.1±1.4	13.4±1.4	12.6±1.3
Day 42	14.8±0.9	15.1±1.1	15.1±1.1	14.6±1.4	13.1±0.9	13.1±1.3	13.4±1.5	12.6±1.3
Day 56	15.0±1.0	15.5±1.2	15.1±1.1	14.5±1.2	13.3±0.8	13.2±1.4	13.6±1.3	12.8±1.2
Day 77	15.2±0.8	15.7±1.1	15.3±1.0	14.4±1.4	13.6±0.8	13.5±1.3	13.8±1.3	12.7±1.3
Day 91	15.4±0.8	15.8±1.4	15.4±1.2	14.4±1.4	13.8±0.9	13.6±1.3	13.9±1.2	12.8±1.3
gain, days -1 to 91	0.94±0.82	0.98±0.45	1.00±0.37	-0.1±0.82 (-110.6%)	1.28±0.51	0.82±0.37 (-35.9%)	0.92±1.02 (-28.1%)	0.34±0.40 (-73.4%)

Data taken from Table IA. pages 84-91 and 191-194 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related; Note: mean body-weight gain computed from individual animal data by the reviewer; percentages of deviation of control body weights were not computed in the Report

C. Food consumption and food efficiency:

Table 3. Food consumption data of female dogs, g/dog/d ± SD

	♀ (N = 5/group)			
mg/kg bw/d	0	10	50	150
Day 0	359±57	379±47	362±38	322±53
Day 7	385±47	373±38	388±26	335±52
Day 14	383±23	381±43	396±8	339±58
Day 21	400±0	391±20	393±15	380±30
Day 28	400±0	400±0	391±21	400±0
Day 35	400±0	400±0	400±0	400±0
Day 42	400±0	400±0	400±0	400±0
Day 49	400±0	395±10	400±0	387±29
Day 56	400±0	400±0	400±0	368±72
Day 63	400±0	392±17	400±0	368±72
Day 72	400±0	392±17	400±0	380±46
Day 80	400±0	400±0	378±49	388±27
Day 91	400±0	400±0	400±0	400±0

Data taken from Table IA. pages 68-83 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

Table 4. Food efficiency data, mean±SD

	♂ (N = 5/group)				♀ (N = 5/group)			
mg/kg bw/d	0	10	50	150	0	10	50	150
Day 7	4.48±4.88	6.99±2.60	9.38±4.42	5.62±1.40	7.88±3.87	7.38±4.53	6.37±3.66	-1.87±5.55**
Day 14	2.86±6.87	-2.15±4.07	2.15±1.96	-1.43±4.09	-0.88±3.20	1.69±4.54	-1.87±7.31	-2.15±7.04
Day 21	2.15±4.09	4.31±7.78	2.16±1.97	4.30±5.89	4.54±6.37	4.19±7.06	8.13±4.45	3.29±3.31
Day 28	1.44±7.42	2.87±6.40	8.61±6.54	-0.00±7.60	-0.46±7.81	0.06±6.24	-0.68±6.33	0.96±13.2
Day 35	-2.16±4.83	-2.15±6.51	-1.43±6.50	5.03±12.08	3.58±4.38	-0.68±4.7	2.17±4.09	2.58±18.0
Day 42	1.43±4.08	0.72±6.88	0.70±9.93	-0.01±9.46	5.72±4.80	-1.52±7.92	0.65±5.46	1.42±3.25
Day 49	5.73±4.08	10.02±13.0	3.58±7.59	0.72±6.41	2.94±8.62	2.30±6.15	-2.90±8.22	-0.10±9.32
Day 56	2.15±9.00	2.15±7.85	-2.88±5.89	-5.75±9.01	3.59±3.58	3.30±7.26	7.89±4.66	4.56±8.41
Day 63	2.86±6.40	-0.71±8.53	8.59±4.80	-1.43±7.42	0.67±5.91	2.20±6.03	0.71±4.68	-4.57±7.39
Day 70	3.59±3.59	5.01±6.97	2.87±3.00	4.29±2.99	7.15±5.05	6.35±11.2	3.58±6.71	-1.05±13.3
Day 77	0.00±4.38	4.29±1.59	-3.58±3.58	-5.73±4.80	3.58±5.06	0.03±8.04	2.87±3.00	0.32±6.45
Day 84	4.28±7.75	-0.71±6.41	4.29±9.92	-4.31±6.42	2.86±6.40	2.91±4.68	-0.35±6.23	0.10±3.71
Day 91	4.31±3.00	3.59±5.67	-0.01±4.39	4.31±4.68	4.31±3.93	0.63±8.23	5.75±6.53	4.40±8.11

Data taken from Table IA. pages 92-95 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

Food consumption of males was not affected; most males consumed the daily rationed amounts of food

during the dosing period. For the females, mean food consumption of the high-dose dogs was lower than the control value during most of the dosing period, more pronounced during the first 24 days of dosing. Mean food efficiency data were highly variable, with large standard deviations. Consequently, there were rarely treatment-related statistically significant findings although the high-dose dogs appeared to have lower food efficiencies when compared to the control animals.

D. Ophthalmoscopic examination: There were no treatment-related effects on the eyes.

E. Blood analyses:

1. Hematology: Table 5

Throughout the study period, statistically significantly decreased values for mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were recorded (assessed at days 41/43 and 93/94) in dogs at 150 mg/kg bw/d. It was stated in the study report that red blood cell morphology showed increased microcytosis and polychromasia at both time intervals in these dogs and increased anisocytosis was also noted in males at 150 mg/kg bw/d on day 41 and in females at 150 mg/kg bw/d on days 43 and 94. Moreover, in males at 150 mg/kg bw/d hemoglobin concentrations were significantly decreased on days 41 and 93 and hematocrit values were reduced on day 93.

Statistically significantly increased platelet counts were observed at 150 mg/kg bw/d (♂ on days 41 and 93; ♀ on day 94). However, assessment of blood clotting parameters did not show any treatment-related effects. At the end of the study an increase in red blood cells was measured in high-dose females only. This isolated finding was considered incidental unrelated to BAS 800 H administered, because it was inconsistent with the mode of action of BAS 800 H. At 50 mg/kg bw/d, significantly decreased MCH was noted on days 41 and 93 and reduced MCV in males and significantly lower values for MCV and MCH were found in females on day 94.

Table 5. Selected hematological and clinical chemistry values, mean±SD

	♂ (N = 5/group)				♀ (N = 5/group)			
mg/kg bw/d	0	10	50	150	0	10	50	150
RBC d41/43	7.18±0.39	6.75±0.38	7.06±0.26	7.08±0.50	7.23±0.33	7.45±0.23	7.41±0.62	8.01±0.56
10^{12}/L d93/94	7.52±0.48	6.86±0.15*	7.62±0.30	7.88±0.50	7.06±0.42	7.30±0.63	7.45±0.42	8.62±0.72**
Hb, d41/43	10.5±0.4	9.8±0.4*	9.7±0.6	**9.0±0.7***	10.5±0.6	10.6±0.2	10.2±0.7	9.9±0.7
mmol/L d93/94	10.7±0.7	9.8±0.4	10.0±0.8	**8.7±0.5****	10.3±0.7	10.4±0.6	10.1±0.7	9.1±0.5
Hct, % d41/43	46.8±1.8	44.8±2.1	44.2±2.9	42.1±3.1	48.7±2.8	49.0±1.4	47.8±3.8	47.3±3.1
d93/94	49.0±2.4	45.5±2.3	46.0±3.4	**41.7±2.1****	47.4±3.4	47.7±2.8	46.8±3.0	44.0±2.0
MCV, fL d41/43	65.2±1.5	66.4±2.9	62.7±2.4	**59.4±2.3****	67.4±2.2	65.8±2.2	64.5±2.0	**59.1±2.4****
d93/94	65.1±1.1	66.3±2.9	**60.4±4.0****	**53.0±2.1****	67.2±2.2	65.5±2.4	**62.8±2.3***	**51.2±3.3****
MCH d41/43	1.46±0.04	1.45±0.06	**1.37±0.05***	**1.27±0.05****	1.44±0.04	1.42±0.04	1.39±0.04	**1.24±0.05****
fmol d93/94	1.43±0.02	1.43±0.05	**1.31±0.09****	**1.10±0.03****	1.46±0.04	1.43±0.06	**1.35±0.04****	**1.06±0.07****
MCHC d41/43	22.4±0.7	21.9±0.24	21.9±0.20	**21.4±0.54***	21.4±0.14	21.6±0.22	21.5±0.44	**21.0±0.16****
,mmol/L d93/94	21.9±0.23	21.6±0.13*	21.7±0.22	**20.8±0.62****	21.8±0.37	21.9±0.21	21.5±0.43	**20.7±0.30****
WBC d41/43	9.87±1.1	11.2±1.45	11.8±1.53	**12.6±1.97**	11.2±1.78	9.9±0.65	12.3±3.32	12.4±3.93
10^9/L d93/94	10.3±0.91	11.7±1.06	11.8±1.72	12.1±1.48	11.4±2.09	10.1±0.98	12.1±1.68	11.6±2.96
Platelets d41/43	309±71	324±24	359±38	447±93*	314±20	307±37	335±27	432±124
10^9/L d93/94	320±64	310±35	373±45	497±116*	322±31	319±59	367±51	533±153**

Data taken from Table IB, pages 96-115 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

2. Clinical chemistry: Table 5

Throughout the administration period statistically significantly increased alkaline phosphatase activities were recorded in males at 150 mg/kg bw/d and in females at 50 and 150 mg/kg bw/d. The activities of other enzymes were not adversely affected by BAS 800 H.

71

Blood chemistry examinations revealed significantly lower total protein and albumin levels at 50 (♂) and 150 (♂♀) mg/kg bw/d throughout the study. Lower albumin levels were also seen in males at 10 mg/kg bw/d. Total bilirubin concentrations were decreased when assessed on days 41/43 (all ♂; ♀ at 50 and 150 mg/kg bw/d), but not at the end of the dosing period on days 93/94. The decreased bilirubin and albumin concentrations in males at 10 mg/kg bw/d were not considered to be treatment-related because the values of both parameters were within or near the lower limit of the historical controls. No test compound-related changes were found in the other blood chemistry parameters examined.

Table 5. Selected hematological and clinical chemistry values, mean±SD

		♂ (N = 5/group)				♀ (N = 5/group)			
mg/kg bw/d		0	10	50	150	0	10	50	150
AP	d41/43	1.51±0.22	1.63±0.36	2.01±0.35	**3.21±1.02***	1.42±0.15	1.66±0.22	2.11±0.48*	**2.71±0.62***
µkat/L	d93/94	1.47±0.38	1.66±0.43	2.17±0.64	**3.69±1.56***	1.49±0.22	1.73±0.34	2.41±0.72*	**3.50±0.81****
ALT	d41/43	0.71±0.12	0.63±0.35	0.41±0.06**	0.44±0.08**	0.46±0.08	0.47±0.07	0.46±0.11	0.48±0.12
µkat/L	d93/94	0.75±0.22	0.60±0.14	0.53±0.14	0.52±0.14	0.59±0.16	0.45±0.09	0.45±0.13	0.57±0.20
Bilirubin	d41/43	3.23±0.24	2.30±0.41**	2.07±0.66*	1.66±0.30*	3.57±0.50	3.53±0.33	2.67±0.44**	2.68±0.54**
µmol/L	d93/94	3.13±0.36	3.23±0.46	2.16±0.42	2.53±0.22	4.15±1.21	3.74±0.55	2.89±0.16	2.69±0.27
T Protein	d41/43	63.7±2.72	60.6±3.55	58.4±1.77*	57.2±1.23**	59.0±1.43	59.1±0.60	59.3±2.91	55.7±1.62*
g/L	d93/94	65.0±2.02	61.7±2.74	60.6±2.51*	58.1±2.18**	62.3±0.88	61.8±1.12	62.1±2.51	57.9±2.42
Albumin	d41/43	36.7±1.77	33.9±1.67	31.9±1.22**	30.4±0.98**	35.3±1.50	35.1±0.81	33.9±2.05	30.5±1.23**
g/L	d93/94	37.1±1.17	33.8±1.76*	32.5±1.86**	30.0±1.10**	36.2±1.07	35.5±0.72	35.5±1.74	30.6±1.66**

Data taken from Table IB. pages 116-142 of Report; AP = alkaline phosphatase; ALT = alanine aminotransferase
* ≤0.05; ** ≤0.01; **bold** values are considered treatment-related

F. Urinalysis: Urinalysis revealed no treatment-related changes.

G. Sacrifice and pathology:

1. Organ weight:

With the exception of slight increase in liver weights of high-dose males, there were no obvious treatment-related effects on organ weights.

Table 6. Selected organ weight values, mean±SD

		♂ (N = 5/group)				♀ (N = 5/group)			
mg/kg bw/d		0	10	50	150	0	10	50	150
BW, g		15400 ±837	15760 ±1419	15520 ±1126	14480 ±1359	12575 ±685	12800 ±913	12575 ±613	12200 ±1992
liver	g	379±22	401±57	407±38	411±35	397±17	383±51	357±39	378±31
	%BW	2.47±0.20	2.54±0.21	2.63±0.19	**2.84±0.17****	2.88±0.26	2.79±0.35	2.55±0.09	2.94±0.25
kidneys	g	70.6±9.7	69.3±5.1	73.7±12.3	76.6±12.1	56.8±5.6	62.0±4.7	60.1±7.4	60.2±5.1
	%BW	0.457 ±0.042	0.443 ±0.051	0.475 ±0.068	0.529 ±0.059	0.411 ±0.044	0.453 ±0.051	0.430 ±0.037	0.469 ±0.043
spleen	g	38.5±9.4	34.2±8.7	35.2±3.2	29.5±2.8	33.1±3.4	38.8±10.4	37.3±5.5	38.2±7.8
	%BW	0.248 ±0.048	0.219 ±0.061	0.227 ±0.013	0.204 ±0.021	0.238 ±0.011	0.280 ±0.065	0.269 ±0.046	0.295 ±0.047
thymus	g	11.8±3.45	8.58±2.17	10.7±1.66	7.37±2.76	11.6±3.61	6.25±2.03*	7.09±2.04*	9.26±3.14
	%BW	0.076 ±0.019	0.054 ±0.011*	0.069 ±0.011	0.051 ±0.017*	0.084 ±0.027	0.045 ±0.015*	0.050 ±0.011	0.072 ±0.023

Data taken from Table IC. pages 146-153 of.Report; * ≤0.05; ** ≤0.01; **bold** values are considered treatment-related

2. Gross pathology: There were no treatment-related gross pathological findings.

3. Microscopic pathology:

Substance-induced microscopic findings were observed in the liver (iron storage), spleen (extramedullary

27

hematopoiesis) and bone marrow (hypertrophy) of male and female dogs at 150 mg/kg bw/d. Iron storage in the liver and kidneys was also observed in one low-dose male and 2 mid-dose males. The authors considered the findings secondary to microcytic hypochromic anemia. The defect in the heme synthesis led to an excess of iron or iron containing intermediate products, which then was intracytoplasmatically stored in liver and spleen cells. Extramedullary hematopiesis and bone marrow hyperplasia are typical findings associated with anemia and are considered a compensatory response. There were no other treatment-related microscopic findings.

Table 7. Selected microscopic findings, number of dogs affected

		♂ (N = 5/group)				♀ (N = 5/group)			
mg/kg bw/d		0	10	50	150	0	10	50	150
Bone marrow (femur)	Hyperplasia					0	0	0	2
Liver	Iron storage			1	3			1	4
Spleen	Iron storage	0	1	2	4	0	0	3	5
	Extramedullary hematopoiesis							1	2
Sternum, with marrow	hyperplasia				2				2
Data taken from Table IC. pages 155-159 of Report; bold values are considered treatment-related									

III. DISCUSSION

1. Authors' conclusions:

"In conclusion, signs of general systemic toxicity, such as impaired body weight data and effects on the hematopoietic system, were seen down to 50 mg/kg BW/day. At the highest dose, a moderate-to-severe anemia was seen as evidenced by numerous impacts to the hematopoietic system with secondary histopathologic findings in liver, spleen and bone marrow.

Therefore, under the conditions of the present study, the no observed effect level (NOEL) and no observed adverse effect level (NOAEL) for male and female Beagle dogs was 10 mg/kg BW/day."

2. Reviewer's comments:

The study was properly conducted and reported. The conclusions of the authors are acceptable.